Analysis of *in silico* stereoelectronic properties of PMD (p-menthane-3-8-diols) and its derivatives to develop a pharmacophore for insect repellent activity

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Introduction

PMD (p-menthane-3-8-diol) is an insect repellent that can be either synthesized chemically or derived directly from the steam distillate residue of the leaves of lemon eucalyptus, *Corymbia citriodora citriodora*. It is one of the few natural products endorsed by the CDC for topical application to protect against mosquitoes [1]. However, no analytical or quantitative structure activity studies or toxicological evaluations of PMD have been reported in the open literature. In our ongoing efforts to understand the mode of action of various insect repellents [2], we have performed a detailed quantum chemical (RHF/6-31G**) based analysis of stereoelectronic properties of PMD and twelve synthetic derivatives. Furthermore, we have also developed a three dimensional feature based pharmacophore for repellent activity of the compounds and compared the features with other known repellents. Our studies with calculated and experimental observations indicate that lower aqueous stabilization (favorable lipophilicity) and larger separation of electrostatic potential energy together with a large localized negative electrostatic potential region by the oxygen atom play a definite role in the repellent activity of these compounds. The generated pharmacophore contains two aliphatic hydrophobic and a hydrogen-bond donor features that mapped well onto the potent compounds but failed to map onto the less potent analogues. The calculated stereoelectronic profiles and the features of the pharmacophore for the PMD analogues should aid design of more effective insect repellents.

Response of female *Aedes aegypti*1 in the contact irritancy assay to unknown chemicals in the laboratory (Table 1)*

Response of female *Aedes aegypti*1 in the contact irritancy assay to unknown chemicals in the laboratory.

Chemical	Concentration (nmoles/cm²)	Number of trials (No. mosq.)	Number escaping (mean ± SE) Treated	Control	Percent escaping[2] (mean ± SE)	p^2
#1	250	6(60)	2.5 ± 0.6	0.3 ± 0.2	42 ± 7	0.0130
#2	250	6(60)	2.2 ± 1.1	0.5 ± 0.2	37 ± 12	0.2965
#3	250	6(60)	2.7 ± 0.5	0.0 ± 0.0	45 ± 7	0.0022
#4	250	6(60)	0.0 ± 0.0	0.3 ± 0.2	4 ± 2	0.4545
#5	250	6(60)	3.5 ± 1.1	0.2 ± 0.2	34 ± 11	0.0043
#6	250	6(60)	2.2 ± 0.8	0.3 ± 0.2	19 ± 8	0.0260
#7	250	6(60)	0.8 ± 0.5	0.2 ± 0.2	6 ± 6	0.4242
#8	250	6(60)	0.0 ± 0.0	0.2 ± 0.2	-2 ± 2	1.0000
#9	250	6(60)	3.0 ± 0.4	0.3 ± 0.2	46 ± 6	0.0022

* Taken from experimental data presented by Dr. K. Chauhan, USDA at the AMCA meeting 2007

Experimental and predicted repellent activity of the compounds in the training set (Table 2)*

Compound#	Experimental P^3	Predicted P^3	Number escaping (mean ±SE) Treated	Number escaping (mean ±SE) Control
#1	0.013	0.013	2.5±0.6	0.3±0.2
#2	0.2965	0.37	2.2±1.1	0.5±0.2
#3	0.0022	0.003	2.7±0.5	0.0±0.0
#4	0.4545	0. 39	0.0±0.0	0.3±0.2
#5	0.0043	0.007	3.5±1.1	0.2±0.2
#6	0.0801	0.044	2.2±0.8	0.3±0.2
#7	0.4242	0.4	0.8±0.5	0.2±0.2
#8	1.0	0.34	0.0±0.0	0.2±0.2
#9	0.0022	0.0037	3.0±0.4	0.3±0.2
10i	NA	0.005	NA	NA
11i	NA	0.002	NA	NA
12i	NA	0.006	NA	NA
13i	NA	72.0	NA	NA

*Experimental P^3 and related data taken from the presentation by Dr. K. Chauhan, USDA at the AMCA meeting 2007

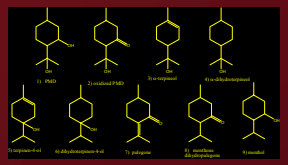
Methodology

3D-QSAR & Pharmacophore Generation: CATALYST software [3] was used. The algorithm treats molecular structures as templates comprising of chemical functions localized in space that will bind effectively with complementary functions on the on the respective binding proteins. The most relevant biological features are extracted from a small set of compounds that cover a broad range of activity.

***Ab Initio* QM calculations:** These calculations were calculated using RHF/6-31G** basis set of Gaussian98 package [4] on a SGI Octane workstation.

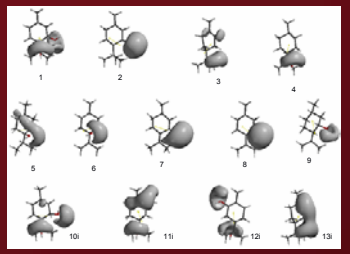
Repellent activity of the PMDs: As shown in Tables and ref [1].

Structure of the PMD analogues*

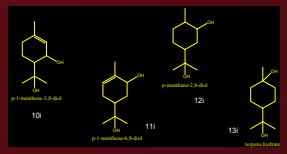


* PMD structures presented by Dr. K. Chauhan, USDA at the AMCA meeting 2007

Polarity directions (shown with arrows) & MEP at -20 kcal/mol of the PMD analogues (polar compounds are more skin irritant than repellent)



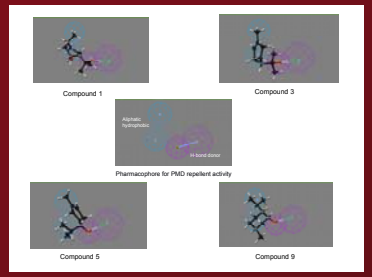
Structure of the New Investigational PMD analogues*



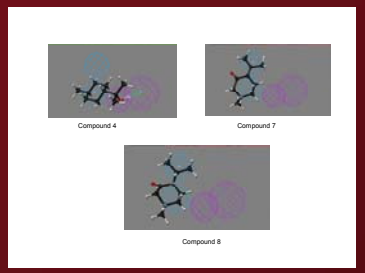
* Investigational PMD structures presented by Dr. K. Chauhan, USDA at the AMCA meeting 2007

RESULTS

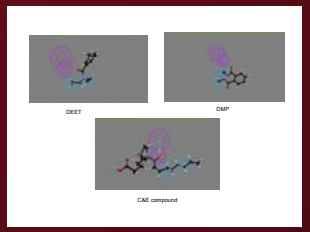
Pharmacophore and its mappings for repellent activity of the PMDs



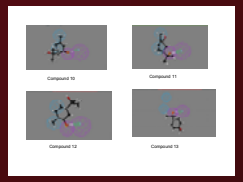
Mapping of the pharmacophore on the PMDs with poor repellent activity



Mapping of the PMD-pharmacophore on other repellents



Mapping of the PMD-pharmacophore on the investigational repellents*



Summary

1. Three dimensional pharmacophore model for insect repellent activity of the PMDs demonstrated a good correlation (R = 0.9) with experimental data.
2. For repellents activity, it appears that the PMDs require a hydrogen bond donor group and two aliphatic hydrophobic features for potent activity. In contrast, the pharmacophoric requirements for DEET are a hydrogen-bond acceptor group and two hydrophobic groups [2]. Interestingly, the PMD - pharmacophore as well as the DEET-pharmacophore [2] map well onto 5-[5-(1-Hydroxy-nonyl)-tetrahydro-Furan-2-yl]-pentanoic acid a recently reported insect repellent, a novel 18-carbon acid, isolated from samples of greasy gaur hair which can also function as a landing and feeding deterrent to mosquitoes [5]. Comparison of PMD-pharmacophore with the DEET pharmacophore clearly indicates a different kind of mechanism of repellent action of the PMD analogues.
3. The model has been cross-validated by statistical CatScramble analysis (95% confidence level).
4. Calculated stereoelectronic profiles of the PMDs are consistent with the pharmacophore model. A large extended electrostatic potential region and weak electrostatic field (hydrophobic) regions appear to favor potent activity of the compounds
5. Three of the four new investigational PMDs map well onto the pharmacophore that are currently being tested.
6. The model is being also used to identify several new potential repellent compounds from virtual screening of the in-house CIS database [6].

References

1. Strickman, D. 2006. PMD (p-menthane-3,8-diol) and quwenling. Chapter 20. In "Insects Repellents, Principles, Methods, and Uses", pp. 347-351.
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